OMSTH

The power of digital PCR – finding a needle in a haystack

Introduction

Digital PCR (dPCR) is a technique where the sample is partitioned into many individual reactions so that either zero, one or more target molecules are present in each reaction. This approach makes it surprisingly easy to detect a single positive against a strong background of negatives, a typical needle in a haystack problem. In such instances, the lower limit of detection excludes standard real-time quantitative PCR (qPCR) as a viable method.

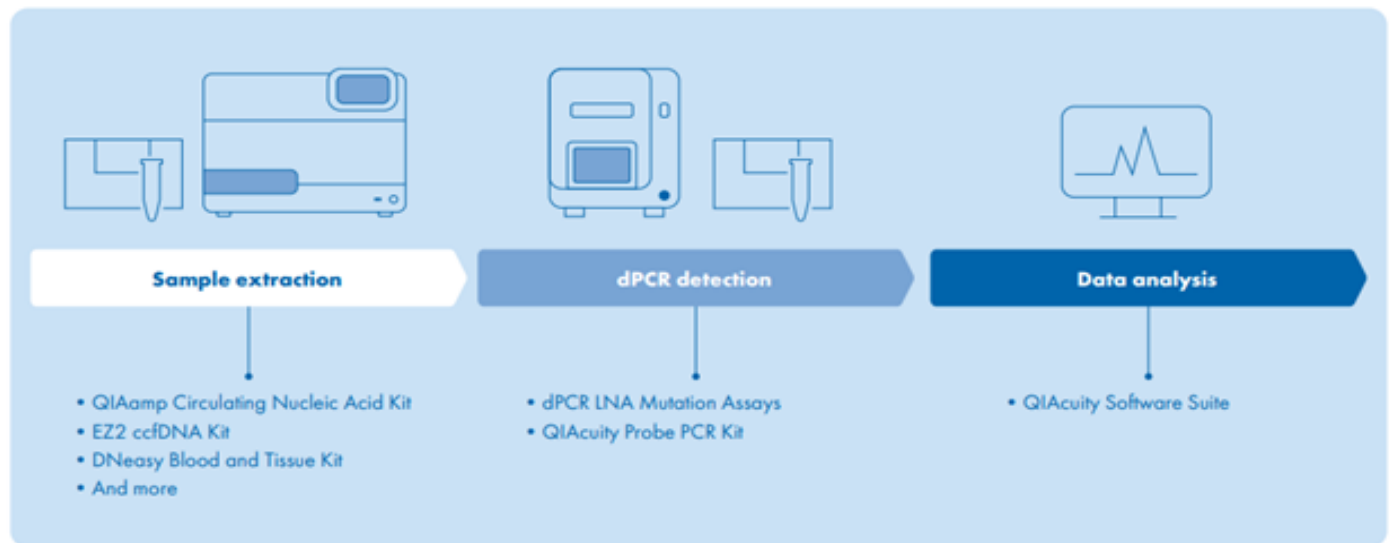Qiagen Digital PCR

Transforming the PCR Experience

If your future is digital, why shouldn't your PCR be, too? Imagine keeping the familiarity and ease of qPCR but gaining the higher sensitivity and precision of digital PCR, without prolonging the time to scientific insight. The QIAcuity, QIAGEN's fully integrated nanoplate-based digital PCR system, has been designed with your research needs and the limitations of the currently available methods in mind. It's time to simplify the transition from qPCR and prepare your critical research applications for the changing landscape of digital PCR.

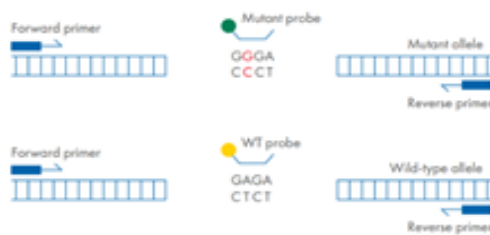## Advantages Over Droplet Digital PCR Technology

The nanoplate-based technology offers significant benefits over digital droplet PCR (ddPCR). These include:
- Fixed partitions prevent variation in size and coalescence
- Sealed nanoplates prevent well-to-well contamination
- Faster readout possible due to simultaneous reading of all partitions of a sample
- User-friendly, familiar plates are easy to pipet, just like for qPCR
- Plates are amenable to front-end automation

Workflow

- Sample extraction
  - QIAamp Circulating Nucleic Acid Kit
  - EZ2 ccfDNA Kit
  - DNeasy Blood and Tissue Kit
  - And more
- dPCR detection
  - dPCR LNA Mutation Assays
  - QIAcuity Probe PCR Kit
- Data analysis
  - QIAcuity Software Suite

Data

The assay contains a primer pair and two probes, a mutant probe and a wild-type (WT) probe, for detecting both mutant and wild-type alleles in the same reaction.
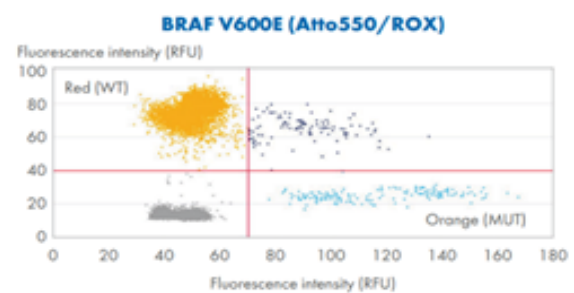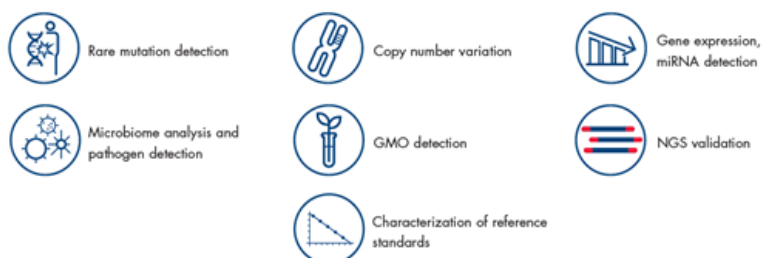

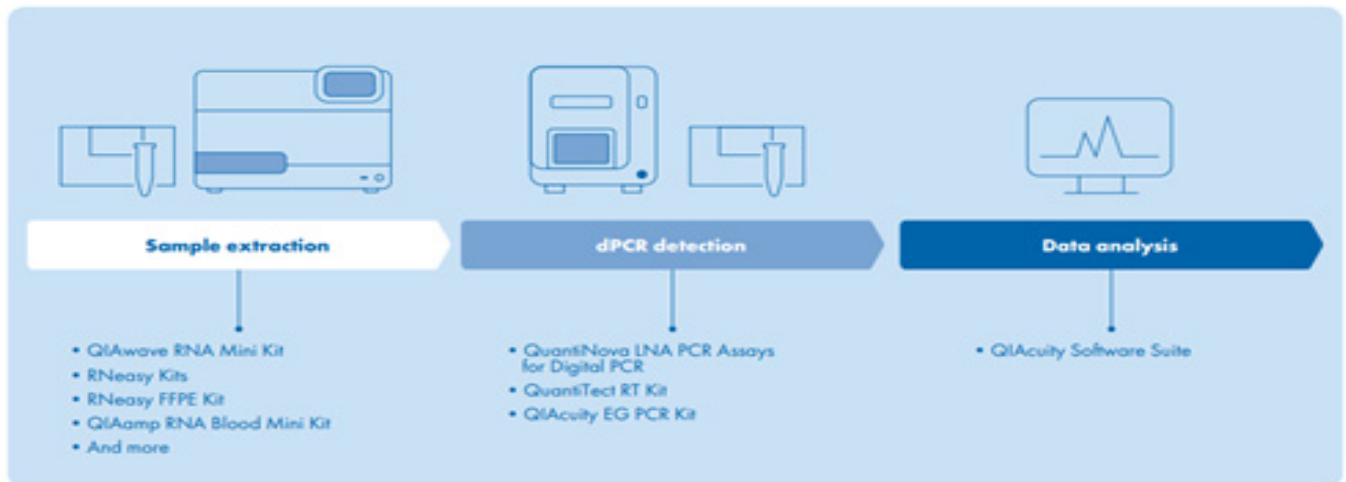A four colors experiment for detecting both EGFR T790M and BRAF V600E in the same well.

Various Applications of Digital PCR

Digital PCR (dPCR) has become increasingly applicable to various research topics over the past decade. The method offers application-specific advantages, such as precision, robustness, speed, and multiplexing capabilities. In this booklet, discover why and how to adapt dPCR to your area of research. Benefit from experimental data and discover complete workflows for optimal dPCR performance in cell & gene therapy, mutation detection, gene expression analysis, microbial detection and many more.
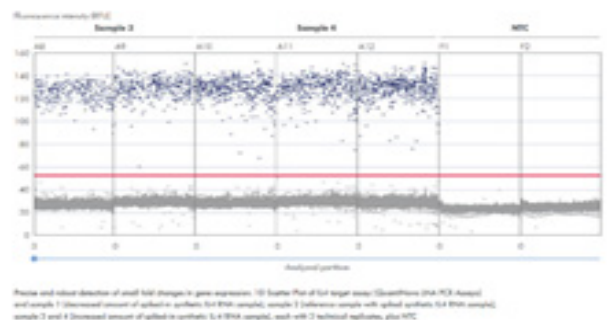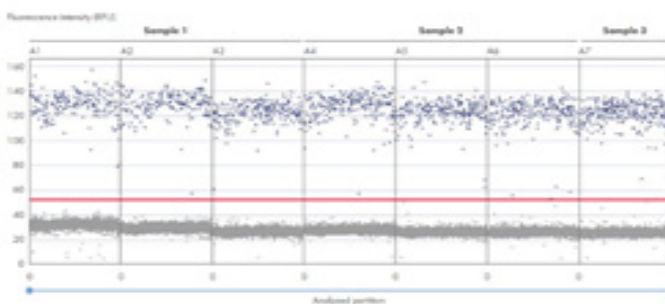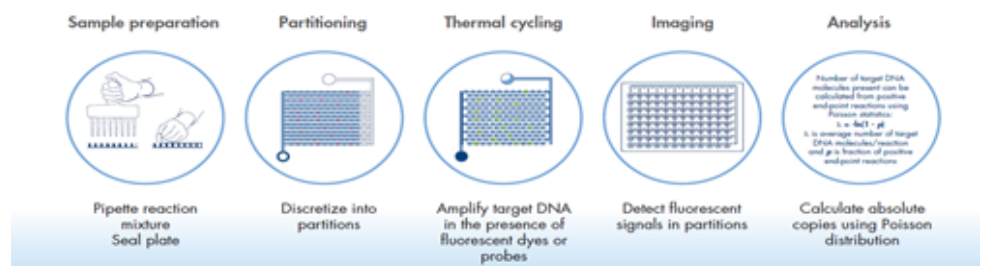
Applications

- Rare mutation detection
- Copy number variation
- Gene expression, miRNA detection
- Microbiome analysis and pathogen detection
- GMO detection
- NGS validation
- Characterization of reference standards

Workflow

Sample extraction	dPCR detection	Data analysis
• QIAwave RNA Mini Kit • RNeasy Kits • RNeasy FFPE Kit • QIAamp RNA Blood Mini Kit • And more	• QuantiNova LNA PCR Assays for Digital PCR • QuantiTect RT Kit • QIAcuity EG PCR Kit	• QIAcuity Software Suite

Data

How it works?

Sample preparation	Partitioning	Thermal cycling	Imaging	Analysis
Pipette reaction mixture Seal plate	Discretize into partitions	Amplify target DNA in the presence of fluorescent dyes or probes	Detect fluorescent signals in partitions	Calculate absolute copies using Poisson distribution

Mutation detection in oncology

Background: Liquid biopsies provide a non-invasive way of retrieving genetic material from tumours and investigating biomarkers and tumour heterogeneity.

Benefits

- High sensitivity in the detection and absolute quantification of rare events and sequence variants

- Detect the presence of a mutation at levels as low as 0.1% in a complex background of wildtype genomic DNA

Gene Expression Quantification

Background: With dPCR, you can quantify gene expression and detect small-fold changes in RNA levels. The high precision of dPCR is beneficial to gene expression profiling, single gene expression studies, investigating gene-regulated pathways or developing diagnostic biomarkers.

Benefits

- In-depth, accurate and robust detection of very small fold changes of mRNA and lncRNA using EvaGreen chemistry
- Superior sensitivity and specificity, thanks to LNA–enhanced primers
- More than 1.3 million pre-designed assays covering all human, mouse, rat mRNA and lncRNA transcripts.